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Chromosomal Studies in Sixteen Species of Indian Pyralid Moths (Pyralidae)

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Abstract. The chromosomes of sixteen Indian Pyralid moths collected from different localities of Bhubaneswar were investigated in male germ line cells using smear preparations, with two species studied using squashes. Among the species investigated, eight had n = 31 (*Antigastra catalaunalis, Crocidolomia binotalis, Dichocrocis punctiferalis, Lamprosema indicata, Lepyrodes neptis, Margorina indica, Maruca testulalis, Pyrausta sanguinalis*), two had n = 30 (*Dichocrocis nilusalis, Galleria mellonella*), three had n = 29 (*Chilotraea infuscatellus, Cnaphalocrocis medinalis, Orthaga exvinacea*) and one species each had n = 27 (*Corcyra cephalonica*), n = 22 (*Tryporyza incertulas*) and n = 12 (*Chilo supressalis*) chromosomes. The trend of evolution in chromosome number tends toward the lower numbers from a modal haploid number n = 31 of the family as well as of the order. All circumstantial evidence indicates the holocentric nature of the chromosomes. The sex-chromosomes in the males are not differentiable, but delayed anaphasic movement of a pair might represent the sex-chromosomes.

Introduction

In spite of intense work carried out during recent years, chromosome studies in Lepidoptera have not made much headway. Of the 130,000 described species of Lepidoptera only 1,300 species have been chromosomally examined, mostly by de Lesse, Suomalainen and Saitoh (c. f. Robinson, 1971). Most work has been concerned with the chromosomes of butterflies, which constitute only 10% of the total species. Moths, forming the majority of species, have been but less attended. Information on chromosome counts of Indian moths is very meagre at present (Gupta, 1964; Rishi, 1973; Nayak, 1975), though some data on a number of Himalayan butterflies are available (Maeki & Ae, 1966; Saitoh & Abe, 1969, 1970 a, b). Of the family Pyralidae, chromosome numbers of 56 species have been reported. The present note deals with the chromosome counts of 16 species of Pyralid moths, of which 12 are reported for the first time.

Materials and Methods

The present investigations were carried out on testes material from late larvae (5th instar) and early pupae collected from their respective

hostplants. Table I gives the account of hostplants and time and place of collection of the different species of Pyralids. The cytological preparations were made by the following procedure: hypotonic treatment of testes (in 0.9% sodium citrate) for about 10 minutes; fixation in aceto-alcohol (1:3) overnight; preparation of smears on pre-warmed albuminised slides using a drop of 45% acetic acid and finally stained in Heidenheins iron haematoxylin.

Table I

List of species examined, with foodplant and date of collection given. All were taken in Bhubaneswar.

Species	Foodplant/Resource	Period of Collection
Antigastra catalaunalis Dup.	*Sesamum indicum*	August, 1976
Chilotraea infuscatellus Sn. (Sugarcane stem borer)	*Saccharum officinarum*	October, 1977
Chilo suppressalis Walk.	*Oryza sativa*	March, 1978
Cnaphalocrocis medinalis Guen.	*Oryza sativa*	October, 1977
Corcyra cephalonica Staint. (Rice moth)	Flour	March, 1976
Crocidolomia binotalis Zell.	*Brassica* sp.	December, 1977
Dichocrocis nilusallis Walk.	*Michelia* sp.	August, 1976
Dichocrocis punctiferalis Guen.	*Ricinus communis*	September, 1977
Galleria mellonella Linn. (Wax moth)	Bee-hive	October, 1977
Lamprosema indicata Fabr.	*Glycin max*	November, 1978
Lepyrodes neptis Cram.	*Nyctanthes* sp.	September, 1978
Margorina indica Saund. (Pumpkin caterpillar)	*Trichosanthes anquina*	August, 1977
Maruca testulalis Gey.	Unidentified	July, 1977
Orthaga exvinacea Hmps. (Mango shoot-webber)	*Mangifera indica*	January, 1976
Pyrausta sanguinalis Linn.	*Ocimum basilicum*	July, 1976
Tryporyza incertulas Walk. (Rice stem borer)	*Oryza sativa*	November, 1978

Observations

Antigastra catalaunalis Dup. (Figs. 1 to 3). A chromosome count in spermatogonial metaphase cells revealed the diploid chromosome number as $2n = 62$. Metaphase I normally showed 31 bivalents. The data are based on 70 metaphase I cells in four specimens. Quite often, one bivalent, presumably the sex-bivalent, appeared to be still undercondensed and was in the form of a ring while others were at their maximal condensation.

A number of cells were observed in which almost all the bivalents had been resolved into univalents except a few which exhibited dumbbell-shape with a distinct notch along the median line. In late anaphase I, all except one bivalent separated into their homologues and passed to their respective poles while components of the laggard still remained on the equatorial region. Metaphase II showed 31 univalents. The haploid chromosome number was, thus, confirmed to be n = 31.

Chilotraea infuscatellus Sn. (Figs. 4 to 6) 2n = 58. Examination of 101 metaphase I cells in 10 specimens showed 29 bivalents in each. The majority of bivalents separated into univalents at anaphase I and passed to their respective poles synchronously. However, in some cases homologues remained still attached to each other by one or two interzonal fibres at their ends and were pulled apart in characteristic V-shapes. As the chromosomes reached the poles of the spindle, they became split into their chromatids before they formed daughter nuclei and much before telophase I. Metaphase II confirmed the haploid number as n = 29.

Chilo suppressalis Walk. 2n = 24. In counts of 46 cells in six specimens, metaphase I cells showed 12 bivalents each. In certain anaphase I cells, a pair of small deeply stained equal sized bodies were visible on the equatorial region of the spindle when all other partners had nearly reached their respective poles. Metaphase II showed 12 univalents (Figs. 7 to 9).

Cnaphalocrocis medinalis Guen. (Figs. 10 to 12) 2n = 58. Metaphase I cells showed 29 bivalents, out of which one very often resolved early into its homologues showing 30 chromosomal elements instead of 29. Metaphase II cells showed 29 univalents. Seventeen cells in two specimens were examined.

Corcyra cephalonica Staint. (Figs. 13 to 15) 2n = 54. Metaphase I cells showed 27 bivalents each. This has been determined by scoring 66 cells in nine specimens. In some cells, early resolution of a number of bivalents into univalents occurred much before the onset of anaphase. A good number of polyploid cells with double the number of bivalents were also noticed. In two such cells almost all the bivalents showed early separation, but the partners of each bivalent remained in close proximity to each other without any actual contact between them. At anaphase I, all bivalents separated into their equal sized homologues simultaneously. Metaphase II cells showed 27 univalents.

Crocidolomia binotalis Zell. (Figs. 16 to 18) 2n = 62. A chromosome count in 59 cells in five specimens established the haploid chromosome number as n = 31. However, a large number of these cells showed 30 bivalents and two univalents, while few others also contained an admixture of bivalents and univalents. Anaphase I cells showed the normal disjunction and synchronous separation of the univalent chromosomes along the spindle. Occasionally, however, one of the bivalents lagged on the equatorial plate. Preparations from one specimen contained a faintly

stained chromosome (smaller than the usual sized elements), which remained off the plate. In anaphase I, this element showed precocious pole-ward movement to only one of the poles and most likely is the supernumerary m-chromosome. Metaphase II showed 31 univalents.

Dichocrocis nilusallis Walk. (Figs. 19 to 21) 2n = 60. Metaphase I cells showed 30 bivalents, one of them being distinctly smaller. This has been determined after examination of 30 cells in four specimens. Certain cells of the cyst showed 31 chromosomal bodies including the two smaller elements lying in pair, these may represent resolved homologues of a bivalent. Some early anaphase I cells were also observed with incomplete separation of the components of some bivalents. Again, in a good number of anaphase I cells of a cyst, components of a bivalent trailed behind in their pole-ward movement. Metaphase II showed 30 spherical univalents.

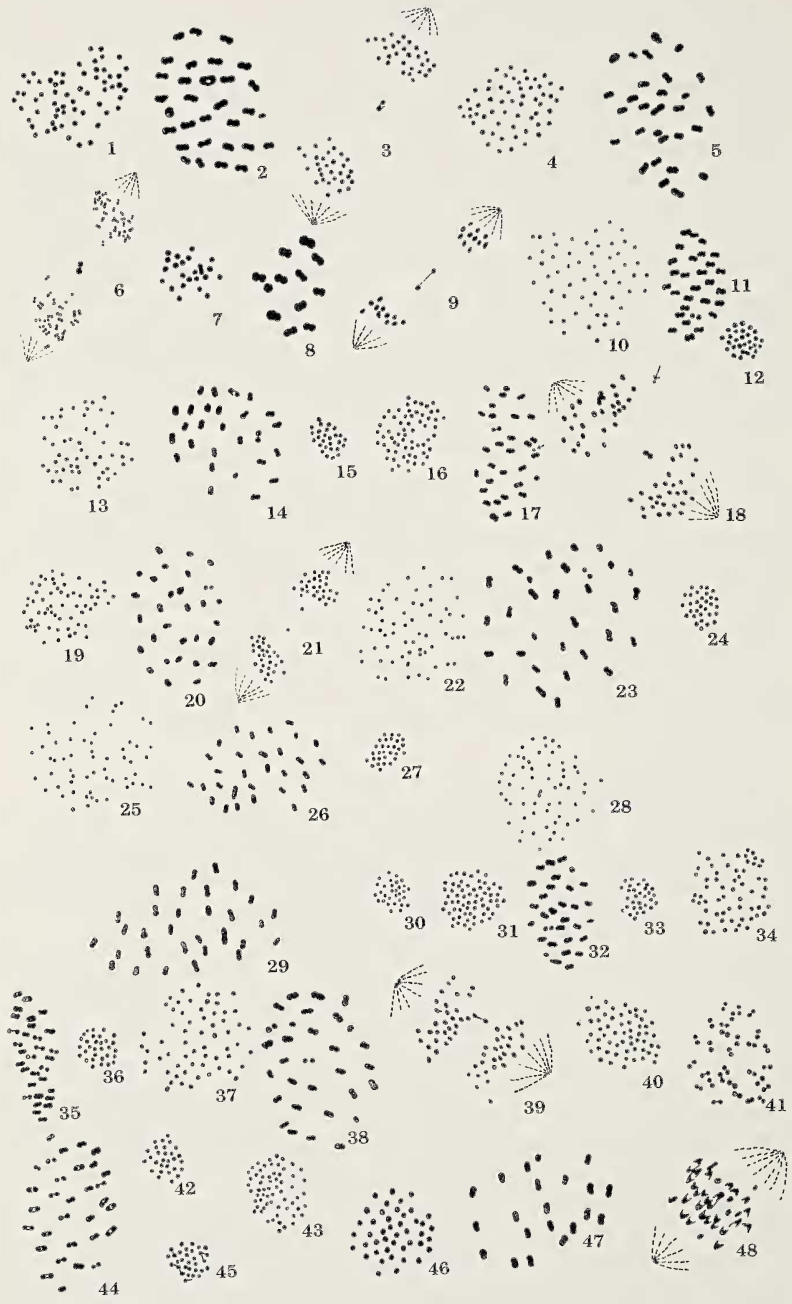
Dichocrocis punctiferalis Guen. (Figs. 22 to 24) 2n = 62. Examination of 126 cells in seven specimens showed metaphase I cells to contain 31 bivalents in each. Anaphase I indicated lagging movement of a bivalent which still remained on the equatorial plate when all others had their homologues passed to their respective poles. Many late anaphase plates also exhibited the laggard on the bridge of the spindle fibres joining the daughter nuclei under formation. Metaphase II cells showed 31 bivalents.

Galleria mellonella Linn. (Figs. 25 to 27) 2n = 60. Examination of 71 cells in nine specimens showed metaphase I cells to contain 30 bivalents each. Eleven tetraploid cells with double the number of bivalents also were observed. Anaphase I was normal, but occasionally presented the atypical lagging behaviour of two of the separating elements. Metaphase II showed 30 univalents.

Lamprosema indicata Fabr. (Figs. 28 to 30) 2n = 62. Metaphase I, as determined by examination of 152 cells in 13 specimens, showed 31 bivalents. One isolated giant pupal cell was observed that was a tetraploid in showing 62 bivalents. Some anaphase I cells showed the lagging behaviour of a pair of small deeply stained equal-sized chromosomal bodies. Metaphase II cells showed 31 univalents.

Lepyrodes neptis Cram. (Fig. 31 to 33) 2n = 62. Examination of 55 metaphase I cells of ten specimens revealed 31 bivalents in each. In a number of cells almost all the bivalents were found to be resolved into their homologues. Several tetraploid cells with double the number of bivalents were also encountered. Some anaphase I cells showed a pair of deeply stained equal-sized bodies (the homologues of a bivalent) on the equatorial region of the spindle when all other chromosomes had almost reached the poles. Metaphase II cells showed 31 univalents.

Margorina indica Saund. (Fig. 34 to 36) 2n = 62. Metaphase I cells showed 31 bivalents as determined by counting 17 cells in three specimens. Preparation from late pupal testes showed the homologues of many bivalents remained in closely placed pairs along with a few intact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Figs. 1 to 3. Spermatogonial metaphase, metaphase I and late anaphase I of *Antigastra catalaunalis.*

Figs. 4 to 6. Spermatogonial metaphase, metaphase I and anaphase I of *Chilotraea infuscatellus.*

Figs. 7 to 9. Spermatogonial metaphase, metaphase I and anaphase I of *Chilo suppressalis.*

Figs. 10 to 12. Spermatogonial metaphase, metaphase I and II of *Cnaphalocrocis medinalis.*

Figs. 13 to 15. Spermatogonial metaphase, metaphase I and II of *Corcyra cephalonica.*

Figs. 16 to 18. Spermatogonial metaphase, metaphase I with m-chromosome and anaphase I with m-chromosome passing to one pole forming an extra plate of *Crocidolomia binotalis.*

Figs. 19 to 21. Spermatogonial metaphase, metaphase I and anaphase I of *Dichocrocis nilusallis.*

Figs. 22 to 24. Spermatogonial metaphase, metaphase I and II of *Dichocrocis punctiferalis.*

Figs. 25 to 27. Spermatogonial metaphase, metaphase I and II of *Galleria mellonella.*

Figs. 28 to 30. Spermatogonial metaphase, metaphase I and II *Lamprosema indicata.*

Figs. 31 to 33. Spermatogonial metaphase, metaphase I and II of *Lepyrodes neptis.*

Figs. 34 to 36. Spermatogonial metaphase, metaphase I and II of *Margorina indica.*

Figs. 37 to 39. Spermatogonial metaphase, metaphase I and anaphase I of *Maruca testulalis.*

Figs. 40 to 42. Spermatogonial metaphase, late abnormal metaphase I cell and II of *Orthaga exvinacea.*

Figs. 43 to 45. Chromosomes of *Pyrausta sanguinalis.*

Figs. 46 to 48. Spermatogonial metaphase, metaphase I and early anaphase I of *Tryporyza incertulas.*

bivalents. Several anaphase I cells showed homologues of a bivalent right on the equatorial region when all others had their separated homologues moved towards poles. Metaphase II showed 31 univalents.

Maruca testulalis Gey. (Figs. 37 to 39) 2n = 62. Examination of 60 metaphase I cells showed 31 bivalents in each. Early separation of a bivalent into its homologues was noticed in some cells. Some anaphase I cells had two of the homologues of a bivalent on the equatorial position when all others had their separated homologues on the march to the poles. Metaphase II cells showed 31 univalents.

Orthaga exvinacea Hmps. (Figs. 40 to 42) 2n = 58. Metaphase I cells showed 29 bivalents as revealed from examination of 85 cells in six specimens. Some of anaphase I cells showed the lagging behaviour of two separating elements of a bivalent. Even at this stage, prior to metaphase II, which followed without interphase, dissociation of separate homologues into chromatids had occurred. Metaphase II cells showed 29 univalents.

Pyrausta sanguinalis Linn. (Figs. 43 to 45) 2n = 62. Metaphase I cells showed 31 bivalents, as determined from 161 cells in 18 specimens. Early separation of a bivalent to its homologues was marked in several cells. Anaphase I was normal. Metaphase II cells showed 31 univalents.

Tryporyza incertulas Walk. (Fig. 46 to 48) 2n = 44. Metaphase I cells showed 22 bivalents in each of the 106 cells counted in six specimens. In one cyst, majority of metaphase I cells showed early separation of a number of bivalents into their homologues. Some cells had all bivalents resolved much before the onset of anaphase I. In early anaphase I cells, the majority of bivalents separated into univalents and migrated to each pole of the spindle synchronously, although some remained attached to each other by interzonal fibres and were pulled apart in characteristic V-shapes, showing bend between arms, giving the impression of the occurrence of a localised centromere. Late movement of homologues of a bivalent was also marked in late anaphase I cells. The chromosomes divided into their respecitive chromatids as they reached the poles. Metaphase II cells showed 22 univalents.

Discussion

The Pyralid karyotype is typical of Lepidoptera in general. Numerous small chromosomes, ill defined meiotic stges, anomalous nature of the centromere and sex chromosomes render karyological analysis obviously difficult. The chromosomes at spermatogonial mitoses are all homomorphic and isodiametric bodies without well defined morphological details. The early meiotic prophase is diffuse, and a clear delineation of leptotene and zygotene is not possible due to the appearance of a characteristic 'synizesis' stage rendering morphology and exact nature of pairing of the chromosomes almost obscure. However, the manifestation of the normal process of lengthwise pairing and parallel conjugation of chromosomes cannot be doubted since in the following pachytene a haploid number of thick paired chromosomes forming bivalents became evident. The diplotene for the most part is 'diffuse' and typical diplotene configuration cannot be seen due to decondensation of the chromosomes which practically recede back to the interphase condition. Thus chiasma analysis is difficult although chiasmata do exist. Chiasma become clear only when some condensation takes place towards late diplotene and diakinesis, when mostly they are terminal or near terminal. Interstitial chiasmata with varying distances from ends producing typical cross configuration are also

observed. Ring configurations with double chiasmata are seen occasionally. In reduction metaphase, the bivalents are evenly distributed and lie well separated from each other. They are dumbbell-shape in a side view and oval in polar view.

Although uniformity in chromosome size is a well-defined feature of Lepidopteran karyotype, slight gradation in size among the chromosomes are nevertheless evident. Direct correlation between chromosome size and number is indicated since species with low chromosome number have larger chromosomes than those with higher number.

While the majority of authors furnish experimental evidence that Lepidopteran chromosomes are holocentric, with diffuse centromeric activity along the entire length of the chromosome (Bauer, 1967; Suomalainen, 1969; Murakami & Imai, 1974), Bigger (1975, 1976) and Rishi (1978), through the use of improved cytological techniques, claim to have demonstrated the presence of a localised centromere in such chromosomes. The current study, however, lends much support to the holocentric nature of Lepidopteran chromosomes. This conclusion is based on behaviour during metaphase I when the spindle fibres are attached to many points on the conjugated chromosomes and no constriction is marked on any of the chromosomes during their anaphasic movement in the majority of species investigated. Indeed during no part of the meiotic cycle is there any evidence of the presence of a localised centromere. The observation is particularly evident in the meiotic prophase where usually the chromosomes are less spiralised and are elongated providing a better display of their linear structure. However, the appearance of a few 'V' shaped chromosomes, similar to centric chromosomes in anaphase I of *C. infuscatellus* and *T. incertulas*, is an exception and the occurrence of a localised centromere, at least on those chromosomes showing the bend, is indicated. Bigger (1975, 1976) demonstrated definite constriction on all the mitotic chromosomes of certain Lepidopteran species—*Pieris brassicae, Pieris napi, Polyommatus icarus* and *Pyronia tithonus*—by employing a new air drying technique combined with ASG banding. According to him at early metaphase the chromosomes of Lepidoptera exhibit a monocentric type of organisation, or at least a part of the diffuse centromere (the primary centromere) which exerts dominant influence over the rest. As metaphase progresses the influence of the primary centromere is either lost or superseded by the combined influence of the rest of the diffuse centromere. Thus, according to Bigger, a primary centromere functions only for a limited part of the meiotic cycle later to be superseded by diffuse centromeric activity along the whole length of the chromosome in Lepidoptera. The primary centromere performs the function of holding the chromatids together at prometaphase and its disappearance at metaphase reflects the evolutionary change from monocentric to holocentric chromosomes. Here we may record Schrader's

(1936, 1939) view of cyclic alternation of centromere from diffuse organisation in late prophase to a strictly localised one at metaphase in *Amphiuma*. Rishi (1978) demonstrated occurrence of a localised centromere in normal diplotene and diakinesis and metaphase I cells of *Trabla vishnu*. Bauer (1967) expressed the opinion that cumulative evidence swung in favour of evolution of holocentric chromosomes from monocentric ones. If so, the few bent chromosomes indicating primary centromeric organisation during present work are the 'relict' providing further supporting evidence in this regard.

Out of the 16 species reported herein, eight species show the haploid number as n = 31 (*A. catalaunalis, C. binotalis, D. punctiferalis, L. indicata, L. neptis, M. indica, M. testulalis* and *P. sanguinalis*), two species have n = 30 (*D. nilusallis* and *G. mellonella*), three species with n = 29 (*C. infuscatellus, C. medinalis* and *O. exvinacea*), one species with n = 27 (*C. cephalonica*), one with n = 22 (*T. incertulas*) and one has n = 12 (*C. suppressalis*). Thus the observed trend appears toward the evolution of lower chromosome numbers which must have been achieved through chromosomal fusion. The familiar range of variation, however, lies between n = 10 as in *Perinephala coronata* (Bigger, 1960) to n = 41 *Haritala ruralis* (Bigger, 1960). Polyploidy as a means of numerical increase of chromosome number in some Lepidopterans, hypothesized by Lorkovic (1941), appears to have been excluded here since the established chromosome numbers do not fall into any of a common number. We share the same view as held by White (1973) and Suomalainen (1969), that

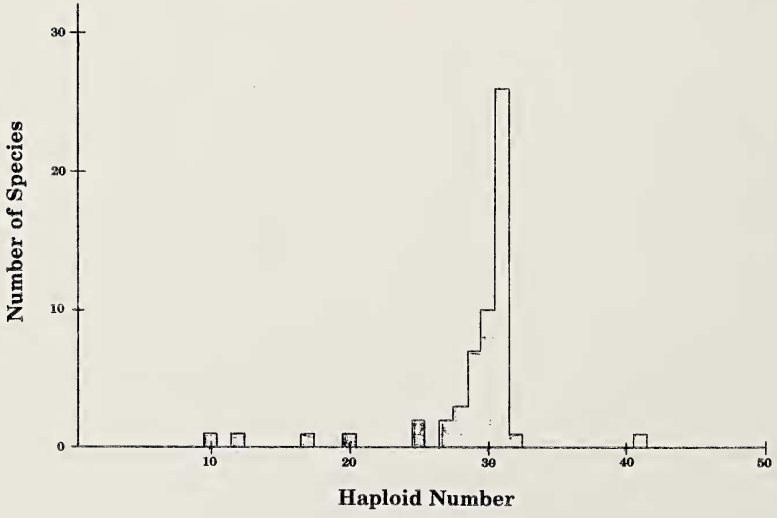

Fig. 49. Histogram showing haploid chromosome numbers in family Pyralidae.

fusion and dissociation are responsible for evolution of chromosome number in Lepidoptera rather than polyploidy, and that fusion is more frequent than fission since there are more species with numbers below 31 than there are ones with numbers above 31. Other factors responsible for an apparent increast in chromosome number include failure of synapsis, early resolution of one or more bivalents to univalents, and the occurrence of one or more supernumerary chromosomes as seen in *D. punctiferalis*. Out of the 56 species of Pyralids cytologically examined so far, the majority (26) of species show a haploid chromosome number n = 31. This is in accord with the standard Lepidopteran karyotype with the modal haploid number n = 31. Since the sex-chromosomes are not differentiable, most of the conclusions in this regard are inferential. In the anaphase I of some species, a pair of chromosomes which show delayed poleward segregation may represent the XX sex-chromosome pair of the male Lepidoptera.

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Literature Cited

BIGGER, T. R. L., 1960. Chromosome numbers of Lepidoptera. Pt. I. Entomol. Gaz. 11: 149-152.

_____, 1961. Chromosome numbers of Lepidoptera. Pt. II. Entomol. Gaz. 12: 85-89.

_____, 1975. Karyotypes of some Lepidoptera chromosomes and changes in their holokinetic organisation as revealed by new cytological techniques. Cytologia 40: 713-726.

_____, 1976. Karyotypes of three species of Lepidoptera including an investigation of B-chromosomes in *Pieris*. Cytologia 41: 216-282.

BAUER, H., 1967. Die kinetische Organisation der Lepidopteren chromosomen. Chromosoma 22: 101-125.

GUPTA, Y., 1964. Chromosomal studies in some Indian Lepdioptera. Chromosoma 15: 540-561.

LORKOVIC, Z., 1941. Die Chromosomenzahlen in der Spermatogenese der Tagfalter. Chromosoma 2: 155-191.

MAEKI, K. & S. A. AE, 1966. A chromosome study of twenty-eight species of Himalayan butterflies (Papilionidae, Pieridae); Spec. Bull. Lepid. Soc. Jap. 2: 107-119.

MURAKAMI, A. & H. T. IMAI, 1974. Cytological evidence for holocentric chromosomes of the silk-worms, *Bombyx mori* and *B. mandarina*, (Bombycidae, Lepidoptera). Chromosoma (Berl.) 47: 167-177.

NAYAK, B., 1975. Studies on the male germinal chromosomes of thirty-one species of moths and butterflies (Lepidoptera). Prakruti-Utkal University Journal-Science: Vol. 12 (1 & 2): 141-150.

RISHI, S., 1973. Chromosome numbers of thirty species of Indian Lepidoptera. Genen Phaenen 16(3): 119-122.

RISHI, S. & K. K. RISHI, 1978. Nature of kinetochore in *Trabla vishnu* Lef. (Lasio-

campidae: Lepidoptera). Abst. 3rd. All India Cong. of Cytology and Genetics, pp. 164.

SAITOH, K. & A. ABE, 1969. Chromosome numbers of some Himalayan butterflies (Lepidoptera: Danaidae, Nymphalidae and Lycaenidae). Kontyu 37(2): 257-258.

_____, 1970a. Chromosome studies in sixteen species of Himalayan butterflies—Nymphalidae and Lycaenidae. Spec. Bull. Lepid. Soc. Jap. 4: 141-152.

_____, 1970b. On the chromosomes of five species of Danaidae from Nepal Himalaya (Lepidoptera, Rhopalocera). Spec. Bull. Lepid. Soc. Jap. 4: 153-158.

SUOMALAINEN, E., 1969. Chromosome evolution in the Lepidoptera. Chromosoma (Berl.) 47: 167-177.

WHITE, M. J. D., 1973. Animal Cytology and Evolution. 3rd ed., London: Cambridge University Press, 1973.